REMARKS

Claims 34-57 are pending in the application. Claims 34 and 35 were withdrawn from consideration.

In the present Amendment, claims 36 and 42 are amended. The amendments to claims 36 and 42 are made in an effort to improve the claim clarity. The amendments are not believed to raise new issues or to require further consideration or search.

No new matter has been introduced. Entry of the Amendment is respectfully requested.

I. Information Disclosure Statements

(1) As an initial matter, it is noted that the Examiner signed Form SB/08 for the IDS filed April 30, 2009, but did not initial the references listed therein. Applicants kindly request the examiner to attach an initialed and signed copy of the form to the next communication to Applicants.

(2) Regarding the IDS filed on July 7, 2006, the Examiner indicated that all the references cited on Form SB/08 were considered, except for JP 62-64802. The Examiner stated that JP 62-64802 was not considered because, according to the Examiner, an English translation of JP 62-64802 was not provided to the Office, and the Examiner contended that it is not apparent that the reference was cited on an International Search Report (ISR).

JP 62-64802 was cited on the ISR. It appears to the undersigned that the Examiner probably looked at the Written Opinion of the ISR (instead of the ISR itself) which mistakenly omitted JP 62-64802 and listed WO 1999/059603 twice. In an effort to expedite prosecution, Applicants re-submit herewith a copy of the ISR. Reconsideration is requested.

Applicants kindly request the Examiner to return an initialed and signed copy of the form PTO/SB/08 submitted with the July 7, 2006 Information Disclosure Statement.

II. Response to Rejections Under 35 U.S.C. § 112, Second Paragraph

Claims 36-57 are rejected under 35 U.S.C. § 112, second paragraph, as being indefinite.

(1) Recitation of "R^2" of claim 36

The Examiner asserts that the recitation "R^2 represents a non-steroidal anti-inflammatory drug residue represented by Z-CO- or hydrogen atom, with the proviso that all R^2's are not hydrogen atoms" renders the claims indefinite.

In response, for further clarity, claim 36 has been amended as suggested by the Examiner to recite that at least one R^2 is a nonsteroidal anti-inflammatory drug residue.

(2) Recitation of "Z-CO-" of claim 36

The Examiner further asserts that the recitation "Z-CO-" renders the claims indefinite because "Z" is not defined in either the Specification and/or the claims. For purposes of examination, the Examiner considers that "Z" refers to a portion of a nonsteroidal anti-inflammatory drug residue, absent the COOH group.

Applicants respectfully traverse.

The instant specification describes on page 6, first paragraph, that "R^2 represents a non-steroidal anti-inflammatory drug residue represented by Z-CO- or hydrogen atom, with the proviso that all R^2's are not hydrogen atoms." The instant specification further describes on page 6, third paragraph, that HOOC-Z represents NSAID before the reaction.

It is respectfully submitted that any person skilled in the art to which the invention pertains reading the instant specification would readily understand that "Z" refers to a portion of a nonsteroidal anti-inflammatory drug residue, absent the COOH group, as correctly understood by the Examiner.

In this regard, for further clarity, claim 36 has been amended to recite that R^2 represents a hydrogen atom or a non-steroidal anti-inflammatory drug residue represented Z-CO-.

(3) Recitation of "a substituent(s)" of claim 42

The Examiner asserts that the recitation "a substituent(s)" in claim 42 renders the claim indefinite. In response, claim 42 has been amended to replace "a substituent(s)" with "one or more substituents."

(4) Conclusion

In view of the claim amendments and remarks, withdrawal of the rejections of claims 36-57 under 35 U.S.C. §112, second paragraph, is respectfully requested.

III. Response to § 103 Rejection based on Tamura and Perioli

Claims 36-57 are rejected under 35 U.S.C. § 103(a) as being unpatentable over EP 1082963 A1 to Tamura et al. ("Tamura"), in view of a newly cited journal publication by Perioli et al. (see PTO-892, Ref. U; "Perioli").

Applicants traverse, and respectfully request the Examiner to reconsider in view of the following remarks and the Declaration evidence submitted herewith.

Independent claim 36 is directed to a hyaluronic acid compound in which a non-steroidal anti-inflammatory drug is bound to hyaluronic acid through a covalent bond, which has a partial structure of a hyaluronic acid disaccharide unit into which the anti-inflammatory drug is introduced, represented by the formula (1) Y-CO-NH-R^1-(O-R^2)$_n$...wherein -HN-R^1-(O-)$_n$ represents a spacer residue in a spacer compound represented by H_2N-R^1-(OH)$_n$ having n numbers of a hydroxyl group...

The Examiner asserts that Tamura discloses each and every element of claim 36, except for the use of a heterobifunctional spacer, such as that instantly claimed. According to

the Examiner, Perioli discloses an aminoalkyl alcohol linker as instantly claimed. See Office Action, at page 9, second and third paragraphs.

The Examiner's rationale is that it would have been obvious to substitute the 1,4-dihydro-1-methylpyridine-3-carboxylate carrier disclosed by Perioli, with the hyaluronic acid carrier disclosed by Tamura, in order to receive the expected benefit as disclosed by Tamura, that conjugation of a NSAID to hyaluronic acid would direct the NSAID drug to a specific site, e.g. the joints, where it is expected to be retained for a long period of time. See Office Action, at page 10, first paragraph.

Applicants respectfully disagree. It is submitted that (1) one of ordinary skill in the art would not be motivated to combine Perioli and Tamura in the manner suggested by the Examiner; and (2) even if the references were somehow combined, the present invention exhibits unexpectedly superior properties as compared to conjugates linked either by a diamide group or a diester group, as discussed in detail below.

First of all, contrary to the Examiner's assertion, one of ordinary skill in the art would not be motivated to substitute the 1,4-dihydro-1-methylpyridine-3-carboxylate carrier disclosed by Perioli, with the hyaluronic acid carrier disclosed by Tamura.

In particular, Perioli relates to a drug design for improving lipophilicity for the purpose of a better blood brain barrier (BBB) penetration and discloses the use of a compound having low molecular weight as a carrier. In contrast, Tamura discloses hyaluronic acid as a carrier. One of ordinary skill in the art would recognize that hyaluronic acid cannot penetrate the BBB, since hyaluronic acid is a high molecular weight substance and is a long chain (polymer) which is composed of repeat structures of a disaccharide unit of N-acetylglucosamine and glucuronic acid.

In this regard, as described in the article entitled "Relationship of Octanol/Water Partition Coefficient and Molecular Weight to Rat Brain Capillary Permeability" (J. Med. Chem. 1980, 23, 682-684), (1) a large molecule is restricted by the BBB and (2) even a molecule having a molecular weight of greater than 400 is extremely restricted in its ability to cross the BBB. See page 682, left column, lines 1-4 and page 684, left column, lines 14-17.

To facilitate the Examiner's review, Applicants submit herewith a copy of the article entitled "Relationship of Octanol/Water Partition Coefficient and Molecular Weight to Rat Brain Capillary Permeability."

Peliori discloses at page 716, right column, lines 19-20, that "[L]ipophilicity is an essential feature for the penetration of a molecule through the BBB." However, hyaluronic acid is an acid having a carboxyl group residue in every hyaluronic acid disaccharide unit and is soluble in water. For purposes of argument, if one of ordinary skill in the art *were* to modify Perioli to use hyaluronic acid as a carrier to improve lipophilicity of a drug, which they would not, the numerous carboxyl groups that exist in every disaccharide unit would be a problem as they have the opposite property of being hydrophilic. Therefore, one of ordinary skill in the art would not be motivated to substitute the carrier of a drug derivative disclosed in Perioli with the hyaluronic acid disclosed by Tamura.

Also, one of ordinary skill in the art would not be motivated to modify Tamura to use the aminoalkyl alcohol linker of Perioli.

According to Tamura, "[a]t the site of administration, either HA [hyaluronic acid] or an HA derivative or a salt thereof which are the active ingredient of HA formulations or the therapeutic for joint diseases exhibit their own efficacies to produce the desired synergism as

they can show their activities without being dissociated or decomposed" (emphasis added). *See* Tamura, paragraph [0148].

On the other hand, Perioli states that "[B]y enzymatic hydrolysis, the active drug is released into the brain and can exert its action" after the BBB penetration. (Emphasis added.) *See* Perioli, at page 716, left column, lines 30-32.

Thus, the derivative of Tamura and the derivative of Perioli act in different ways, i.e., the derivative of Tamura remains as a conjugate, while the conjugate of Perioli is cleaved and the drug is released from the carrier to act on the brain. Applicants submit that one of ordinary skill in the art would not be motivated to use the cleavable conjugate of Perioli wherein a drug is easy to release, in order to treat joint diseases as disclosed in Tamura.

Furthermore, the present invention exhibits unexpected and surprising properties by using a specific combination of the two types of bonding in a compound comprising NSAIDs, hyaluronic acid and a spacer.

The effect of the present invention is shown in Example 47 (Fig. 8) of the present application. In Example 47, the results show that a solution of a compound of the present invention in which the compound has a specific combination of two types of bonding (i.e., hyaluronic acid is bound to an amino alcohol via an amide bond and the amino alcohol is bound to diclofenac via an ester bond) exhibited a remarkable analgesic effect.

In an effort to advance the prosecution, Applicants provide herewith an executed Declaration by Mr. Kenji MIYAMOTO, Mr. Yousuke YASUDA and Mr. Keiji YOSHIOKA providing additional experiments to further demonstrate the unexpectedly superior properties of the claimed invention.

It is respectfully submitted that Example 47 (Fig. 8) of the present application has already shown unexpected and significant effects of the present invention. The experiments in the Declaration are not experiments for elucidating effects of the present invention after filing of the present application. Those additional experiments in the Declaration are submitted in an effort to demonstrate the repeatability of the claimed invention, and to further confirm the effects of the present invention.

As shown in the executed Declaration, three compounds were prepared and tested. In the graphs of the executed Declaration:

- (Control) represents the group of rats treated with PBS
- (Synthesis Example 1 - The present invention) represents the group of rats treated with the compound of the present invention (the combination of an amide bond and an ester bond). Namely, hyaluronic acid is bound to aminoethanol via an amide bond, and aminoethanol is bound to diclofenac via an ester bond.
- (Synthesis Example 2 - C2 diamide Group) represents the group of rats treated with a diamide compound. Namely, hyaluronic acid is bound to diaminoethane via an amide bond, and diaminoethane is bound to diclofenac via an amide bond.
- (Synthesis Example 3 - C2 diester Group) represents the group of rats treated with a diester compound. Namely, hyaluronic acid is bound to ethyleneglycol as a spacer via an ester bond, and ethyleneglycol is bound to diclofenac via an ester bond.

The data in the pain score graph as well as the data in the weight loading graph of the executed Declaration show that the analgesic effect of the compound of the present invention (Synthesis Example 1) exhibited unexpectedly superior properties, i.e., consistent and continued improvement in analgesic effect, lower pain score and higher weight loading rate, as compared to the properties exhibited by the C2 diester Group (Synthesis Example 3) and the C2 diamide Group (Synthesis Example 2).

Because the presently claimed hyaluronic acid compound provides unexpectedly superior results in its analgesic effect, such unexpected properties are evidence of the non-obviousness of the claimed invention.

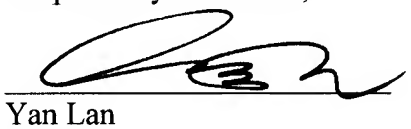
In view of the above, the present claims are not obvious over Tamura in view of Perioli. Reconsideration and withdrawal of the present §103(a) rejection are respectfully requested.

IV. Conclusion

In view of the above, reconsideration and allowance of this application are now believed to be in order, and such actions are hereby solicited. If any points remain in issue which the Examiner feels may be best resolved through a personal or telephone interview, the Examiner is kindly requested to contact the undersigned at the telephone number listed below.

The USPTO is directed and authorized to charge all required fees, except for the Issue Fee and the Publication Fee, to Deposit Account No. 19-4880. Please also credit any overpayments to said Deposit Account.

Respectfully submitted,



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Date: August 25, 2010

Table II. Spiro[tetralin-1,3'-pyrrolidine] Derivatives 2a-n

compd	formula[a]	yield,[b] %	mp, °C	writhing test, mg/kg po	hot-plate test: ED_{50}, mg/kg po[g]
2a	$C_{19}H_{18}NCl$[c]	79	164-176	30, ++++	13.3 (10.5-16.7)
2b	$C_{14}H_{20}NCl$[c]	81	202-210	30, ++++	12.1 (8.4-14.3)
2c	$C_{17}H_{21}NO_4$[d]	85	182-189	30, ++	48.6 (30.3-57.6)
2d	$C_{18}H_{23}NO_4$[d]	92	132-136	100, +++	ND[f]
2e	$C_{14}H_{20}NOCl$[c]	90	174-192	100, +	ND[f]
2f	$C_{19}H_{23}NO_4$[d]	92	147-151	100, ++	ND[f]
2g	$C_{14}H_{20}NOCl$[c]	78	162-169	100, +	ND[f]
2h	$C_{18}H_{23}NO_4$[d]	85	129-135	30, ++	>50
2i	$C_{13}H_{18}NOBr$[e]	60	230-232	30, ++	>50
2j	$C_{14}H_{20}NOBr$[e]	88	255-259	100, +	ND[f]
2k	$C_{13}H_{18}NOBr$[e]	39	195-203	100, ++	ND[f]
2l	$C_{14}H_{20}NOBr$[e]	50	218-230	30, ++	>50
2m	$C_{13}H_{18}NOBr$[e]	67	258-261	100, ++	ND[f]
2n	$C_{14}H_{20}NOBr$[e]	52	182-193	30, ++	42.8 (35.6-49.4)
morphine hydrochloride				10, ++++	1.2 (0.9-1.5)
profadol				10, ++++	3.9 (2.5-5.9)

[a,b] See corresponding footnotes in Table I. [c] Isolated as the hydrochloride salt and recrystallized from ethanol-ether. [d] Isolated as the fumarate salt and recrystallized from ethanol. [e] Isolated as the hydrobromide salt and recrystallized from ethanol. [f] Not determined. [g] Confidence limits in parentheses.

Evaporation of the solvent afforded a yellow oil, which was distilled in vacuo to afford the appropriate spiro[tetralin-1,3'-pyrrolidine] as a colorless oil. Bases were converted immediately to their hydrochloride or fumarate salts.

Synthesis of *N*-Methylspiro[tetralin-1,3'-pyrrolidines]. The appropriate spiro[tetralin-1,3'-pyrrolidine] (1.1 g, 0.005 mol), HCO_2H (2.5 mL), and HCHO (37%, 1.0 mL) were heated together on a water bath for 7 h. After evaporation of the solution to dryness, the residual oil was dissolved in 5% HCl, washed with ether, basified with 10% NaOH, extracted with ether, and dried ($MgSO_4$). After evaporation of the solvent, the residual oil was distilled in vacuo to give the corresponding *N*-methyl derivative as a clear, colorless oil. The free base was converted to either the hydrochloride or fumarate salt (see Table II).

O-Demethylation of Compounds 2c-h. As a general procedure, the appropriate spiro[methoxytetralin-1,3'-pyrrolidine] (0.01 mol) was refluxed under nitrogen for 2 h at 125 °C in 48% aqueous hydrobromic acid (25 mL). The resulting yellow-brown solution was evaporated to dryness under nitrogen, and the residue was taken up in absolute ethanol. On addition of ether and refrigeration, buff crystals of the crude O-demethylated product were obtained. Recrystallization from ethanol-ether afforded a purer product (see Table II).

Pharmacology. Analgesia was determined by the acetic acid writhing test[15] in groups of six mice. Each group was dosed orally with either vehicle ("Dispersol") or compound under test and injected intraperitoneally 30 min later with dilute acetic acid (0.4 mL, 0.25%). The total number of writhes was recorded, and the protection afforded was expressed as a percentage of control values according to the following scale: ++++, 100% inhibition; +++, 75-99% inhibition; ++, 50-74% inhibition; +, 25-49% inhibition. Compounds showing 50% or more inhibition at 30 mg/kg in the above test were also tested for analgesia in mice by the hot-plate[16] method (see Table II).

Acknowledgment. We gratefully acknowledge the support of Imperial Chemical Industries in carrying out and releasing results of the biological testing and the Pharmaceutical Society of Great Britain for providing a research grant for one of us (R.S.).

Relationship of Octanol/Water Partition Coefficient and Molecular Weight to Rat Brain Capillary Permeability

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The rat brain capillary permeability coefficient was determined for 27 compounds. The relationship of permeability to octanol/water partition coefficient and molecular weight was found to be predictable for drugs with molecular weights less than 400.

It is generally believed that the blood-brain barrier (BBB) is restrictive for small molecules at capillary endothelial cells and for large molecules at the interendothelial tight junctions. Although a great deal has been learned about the effects of BBB physiology on the passage of electrolytes and hydrophobic nonelectrolytes, a limited amount of information that correlates lipophilicity, molecular size, and the ability to cross the BBB has been published.[1-3]

We report the brain capillary permeability coefficient (P_c) determined in ether-anesthetized rats for 27 compounds for which the octanol/water partition coefficients are known.

Experimental Section

Isotopes. ^{14}C-labeled urea, creatinine, 5-fluorouracil, sodium ascorbate, and sucrose, ^{3}H-labeled water, glycerol, and galactitol, and $^{24}NaCl$ were purchased from New England Nuclear Corp. and/or Amersham-Searle, Inc. Radiopurity was satisfactory by manufacturer's specifications. ^{14}C-Labeled dianhydrogalactitol, dibromodulcitol, 1,3-bis(2-chloroethyl)-1-nitrosourea (BCNU), 1-(2-chloroethyl)-3-cyclohexyl-1-nitrosourea (CCNU), 1-(2-chloroethyl)-3-(2,6-dioxo-3-piperidyl)-1-nitrosourea (PCNU), *N*-(1-methylethyl)-4-[(2-methylhydrazino)methyl]benzamide monohydrochloride (procarbazine), Baker's antifol, adriamycin,

(1) W. H. Oldendorf, *Proc. Soc. Exp. Biol. Med.*, 147, 813 (1974).
(2) V. A. Levin, H. Landahl, and M. A. Freeman-Dove, *J. Pharmacokinet. Biopharm.*, 4, 499 (1976).
(3) S. I. Rapoport, K. Ohno, and K. D. Pettigrew, *Brain Res.*, in press.

0022-2623/80/1823-0682$01.00/0

Table I. Rat Brain Capillary Permeability Coefficients (P_c)

compd no.	compd	N	M_r	log P	$P_c \times 10^{-6}$ cm/s
1	^3H_2O	13	18	−1.15	200
2	^{24}Na	9	58	−2.95	0.4
3	[^{14}C]urea	28	60	−2.80	0.82
4	[^{3}H]glycerol	10	92	−1.75	12
5	[^{14}C]creatinine	4	113	−1.77	0.28
6	5-fluoro[^{14}C]uracil	4	130	−0.95	1.7
7	[^{14}C]dianhydrogalactitol	6	150	−1.29	2.5
8	[^{14}C]metronidazole	11	171	−0.16	14
9	[^{14}C]ascorbate	5	176	−4.04	1.3
10	[^{3}H]galactitol	9	182	−3.10	0.39
11	[^{14}C]misonidazole	5	185	−0.37	10
12	[^{14}C]ftorafur	7	200	−0.48	6.4
13	[^{14}C]BCNU	2	214	1.54	154
14	[^{14}C]procarbazine	4	221	0.06	19
15	[^{14}C]CCNU	3	234	2.83	100
16	[^{14}C]pyrimethamine	4	249	2.69	120
17	[^{14}C]PCNU	5	263	0.37	11
18	DDMP	6	269	2.82	150
19	[^{14}C]DDEP	2	284	3.19	110
20	[^{14}C]dibromodulcitol	7	308	−0.29	1.9
21	[^{14}C]spirohydantoin mustard	7	315	2.47	29
22	[^{14}C]sucrose	3	342	−3.67	0.12
23	Baker's [^{14}C]antifol	2	398	−2.46	0.18
24	[^{14}C]adriamycin	4	543	−0.10	<0.014
25	[^{3}H]epipodophylotoxin	14	657	2.80	0.20
26	[^{3}H]vincristine	12	825	2.80	0.64
27	bleomycin	4	1400	−3.3	<0.014

misonidazole, ftorafur, and spirohydantoin mustard, and [^{3}H]-vincristine were supplied by Dr. Robert Engle (Chemical Resources Section, National Cancer Institute). Radiopurity and chemical purity were determined before use by thin-layer chromatography (TLC). Adriamycin and vincristine were repurified by TLC immediately before use (98% radiopurity). [^{14}C]Metronidazole was generously supplied by Dr. Rothwell Polk (Searle Laboratories). Radiopurity exceeded 98% by TLC. [^{3}H]Epipodophylotoxin (VM-26) was generously supplied by Dr. R. Dorrien Ven (Sandoz, Inc.). A radiopurity of 98% was confirmed by TLC. [^{14}C]Pyrimethamine and 2,4-diamino-5-(3′,4′-dichlorophenyl)-6-[^{14}C]methylpyrimidine ([^{14}C]DDMP) were generously supplied by Dr. Charles Nichol (Wellcome Research Laboratories).

Nonradioactive Compounds. Bleomycin was a gift of Dr. Stanley Crooke (Bristol Laboratories). Chemical quantification of bleomycin from plasma and tissue samples was performed by Dr. James Strong (Baylor College of Medicine, Houston).[4]

DDEP [2,4-diamino-5-(3′,4′-dichlorophenyl)-6-ethylpyrimidine] was supplied by Dr. Charles Nichol (Wellcome Research Laboratories). Chemical quantification of plasma and tissue levels of DDEP were performed by Ellen Levin.[5]

Octanol/Water Partition Coefficients. The value of spirohydantoin mustard was calculated using π constants.[6] Partition coefficients for [^{3}H]glycerol, [^{14}C]creatinine, and ^{57}Co-bleomycin were determined in our laboratories using the techniques of Hansch.[7] Other values have been published.[8]

Brain Capillary Permeability Measurements.[2,9] For capillary permeability measurements, male Fisher 344 rats weighing 160 to 220 g were anesthetized with ether; isotopes were injected intravenously and blood samples were taken from the femoral artery at different times up to 6 min (to calculate the plasma drug or tracer integral). Rats were sacrificed by decapitation, and the heads were immersed in liquid nitrogen for 45 s. The brain was removed and both cortical and subcortical tissue sections were taken; care was exercised not to include large cerebral vasculature. For radioactivity measurements, tissue and plasma samples were placed in tared scintillation vials, reweighed, and digested with a tissue solubilizer, after which a toluene base fluor was added. For chemical analysis, tissue was placed into tared vials, reweighed, and frozen at −60 °C until analyzed.

The formula used to compute the capillary permeability coefficient, P_c, is shown in eq 1,[2,9] where the tracer distribution,

$$P_c = (DS/t)0.28(ICD)(BV)^{-1/2} \quad (1)$$

DS, over time in seconds is as shown in eq 2. In eq 2, C = cpm/g

$$DS/t = 0.93[C - (C)(PW)](AUC)^{-1/2} \quad (2)$$

of tissue, AUC (the area under the plasma curve during the experimental period) = cpm·min/g of plasma, PW = fractional tissue plasma water volume (mL/g), ICD = the intercapillary distance (cm), and BV = the fractional brain blood volume (mL/g). Values for PW, ICD, and BV for rat brain were determined previously.[2]

Results and Discussion

If the mechanism of nonelectrolyte permeation through capillary endothelial cells is similar to permeation into bulk lipid phases,

$$P_c \propto KD \quad (3)$$

where K is the membrane/water or lipid/water partition coefficient and D is the diffusion coefficient.[10] Because the relationship of the diffusion coefficient to molecular weight, M_r, in bulk solvents for small molecules (M_r < 1000) is[11,12]

$$D(M_r)^{-1/2} \approx \text{constant} \quad (4)$$

it follows that

$$P_c \propto K(M_r)^{-1/2} \quad (5)$$

(4) A. Broughton and J. E. Strong, *Cancer Res.*, 35, 1418 (1976).
(5) E. M. Levin, R. B. Meyer, Jr., and V. A. Levin, *J. Chromatogr.*, 158, 181 (1978).
(6) G. W. Peng, V. E. Marquez, and J. S. Driscoll. *J. Med. Chem.*, 18, 846 (1975).
(7) A. Leo, C. Hansch, and D. Elkins, *Chem. Rev.*, 71, 525 (1971).
(8) C. Hansch and A. Leo, "Substituent Constants for Correlation Analysis in Chemistry and Biology", Wiley Interscience, New York, 1979.
(9) V. A. Levin, M. S. Edwards, and A. Byrd, *Int. J. Radiat. Oncol. Biol. Phys.*, 5, 1627 (1979).
(10) J. M. Diamond and E. M. Wright, *Annu. Rev. Physiol.*, 31, 581 (1969).
(11) W. D. Stein, "The Movement of Molecules Across Cell Membranes", Academic Press, New York, 1967.
(12) W. R. Lieb and W. D. Stein, *Curr. Top. Membr. Transp.*, 2, 1 (1971).

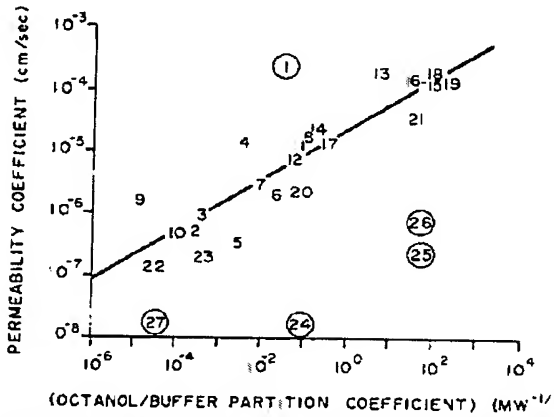

Figure 1. A plot of $P(M_r)^{-1/2}$ vs. P_c for the 27 compounds studied, fit by the method of least squares. The data are plotted as numbers that correspond to the list in Table I. Circled values were not used to compute the best fit line.

This conclusion has been reached by others,[10,11,12] although Collander concluded that eq 6 was a better fit for compounds with molecular weights between 70 and 480.[13]

$$P_c \propto (K_{oil})^{1.32}(M_r)^{-1.5} \quad (6)$$

As a reasonable and first approximation, we evaluated the relationship of P_c to $P(M_r)^{-1/2}$, where P is the octanol/water partition coefficient. Table I lists the molecular weight, log P, and P_c used in this analysis. Figure 1 is a plot of P_c vs. $P(M_r)^{-1/2}$. The line was fit by the method of least squares to 22 of 27 data points. For molecular weights below 400, the line in Figure 1 was fit to eq 7 with

$$\log P_c = -4.605 + 0.4115 \log[P(M_r)^{-1/2}] \quad (7)$$

an SE of estimate = 0.0431, SD of slope = 0.0423, $r = 0.91$, and $n = 22$. Of the five molecules not included, four (bleomycin, adriamycin, vincristine, and epipodophylotoxin) have molecular weights greater than 400, are extremely restricted in their ability to cross the BBB, and are considered to be excluded molecules.[2,9,14] Tritiated water was not included because other factors influence its membrane permeation.

The fact that epipodophyllotoxin and vincristine have permeability coefficients of 2.0×10^{-7} and 6.4×10^{-7} cm/s, respectively, yet are among drugs that do not cross the BBB[9,15] can be rationalized in two ways. First, the radioimpurities associated with these drugs may be smaller, and the more polar impurities may cross brain capillaries to a greater extent than the parent compounds. Second, because of high log P values (2.8), these drugs may penetrate and distribute into but not through brain capillary endothelia. In both cases, this would amount to a small net flux sufficient to produce the observed permeability. We support the second hypothesis because uptake of these labeled compounds over several hours did not indicate significant levels in rat brain.[14,15]

While it would be useful to compare a homologous series of compounds with different log P and molecular weights, it was not possible to do so. Only commercial radiolabeled molecules and labeled anticancer drugs supplied by the Chemical Resources Section, Division of Cancer Treatment, National Cancer Institute, were available to us. Nevertheless, the data are sufficient to derive useful insight into the physical criteria for passive BBB transport.

We draw two conclusions from this study. First, below a molecular weight of 400, increasing lipophilicity will improve P_c. For example, a molecule with $M_r = 400$ and $P = 1$ has a calculated P_c of 7.2×10^{-6} cm/s; for the same molecular weight and $P = 100$, however, P_c increases by nearly sevenfold to 4.8×10^{-5} cm/s. Second, although the absolute cutoff for "significant" BBB passage—regardless of lipophilicity—cannot be stated with certainty from the current study, it is clearly above 400 and below 657 daltons.

These studies have important implications for the design of psychotropic drugs, anticonvulsants, and brain tumor chemotherapeutic agents. The ability of an anticancer drug to cross the BBB is empirically associated with increased activity against CNS tumors when compared with like compounds that do not have this ability. BBB passage alone, however, is an insufficient criterion for antitumor activity. Plasma pharmacokinetics, rate and site of drug biotransformation, tumor capillary to cell transport and blood flow, and the mode of action of a drug are some of the factors that will modulate CNS antitumor activity.[16] Clearly, a logical step to developing better therapy and new therapeutic agents will be an understanding of physical transport factors, such as molecular size and lipophilicity, that influence brain capillary permeability.

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